--This invention was made with government support under Contract Nos. AI33292, AI27742, AI35168 and AI25541 by the National Institutes of Health. The government has certain rights in the invention.--

Please amend the paragraph starting at page 11, line 2, to read as follows:

Figures 10A and 10B illustrate the amino acid residue sequences of variable heavy (V_H) domains of Fabs binding to gp120. Seven distinct groups have been identified as described in Example 9a based on sequence homology. Identity with the first sequence in a group is indicated by dots. The Fab clone names are indicated in the left hand column. The corresponding SEQ ID Nos are indicated in the right hand column. The sequenced regions from right to left are framework region 1 (FR1), complementary determining region 1 (CDR1), framework region 2 (FR2), complementary determining region 2 (CDR2), framework region 3 (FR3), complementary determining region 3 (CDR3), and framework region 4 (FR4). The five amino-terminal residue sequence beginning with LEQ arises from the VH1a while the 5 amino-terminal residue sequence beginning with LEE arises from the VH3a primers. The b11 and b29 sequences are very similar to the b3 group and could be argued to be intraclonal variants within that group; they are placed in their own group because of differences at the V-D and D-J interface.

Please amend the paragraph starting at page 11, line 24, to read as follows:

Figures 11A and 11B illustrate the amino acid residue sequences of variable light (V_L) domains of Fabs binding to gp120. Refer to Figure 10 for the description of the figure and to Example 9b for analysis of the sequences.

Please amend the paragraph starting at page 11, line 29, to read as follows:

Figures 12A and 12B illustrate the amino acid residue sequences of V_L domains from Fabs binding to gp120 and generated by shuffling the heavy chain from clone b12 against a library of light chains (H12-LCn Fabs) as described in Example 10. Note that the new V_L sequences have designated clone numbers that do not relate to those numbers from the original library. The unique sequences are listed in the Sequence Listing from SEQ ID NO 114 to 122. The new V_L domain sequences are compared to that of the original clone b12 V_L sequence.

Please amend the paragraph starting at page 12, line 5, to read as follows:

Figures 13A and 13B illustrate the amino acid residue sequences of V_H domains from Fabs binding to gp120 and generated by shuffling the light chain from clone b12 against a library of heavy chains (L12-HCn Fabs) as described in Example 10. Note that the new V_H sequences have designated clone numbers that do not relate to those numbers from the original library. The unique sequences are listed in the Sequence Listing from SEQ ID NO 123 to 132. The new V_H domain sequences are compared to that of the original clone b12 V_H sequence.

Please amend the paragraph starting at page 14, line 10, to read as follows:

Figure 18A and 18B illustrate the amino acid residue sequences of variable heavy (V_H) domains of Fabs binding to gp41. The Fab clone names are indicated in the left hand column. The heavy chain sequences of the five Fabs individually designated DL 41 19, DO 41 11, GL 41 1, MT 41 12 and SS 41 8 have been assigned the respective SEQ ID Nos 142, 143, 144, 145 and 146. The sequenced regions from right to left are framework region 1 (FR1), complementary determining region 1 (CDR1), framework region 2 (FR2), complementary determining region 2 (CDR2), framework region 3 (FR3), complementary determining region 3 (CDR3), and framework region 4 (FR4).

Please amend the paragraph starting at page 14, line 23, to read as follows:

Figure 19A and 19B illustrate the amino acid residue sequences of variable light (V_L) domains of Fabs binding to gp41. Refer to Figure 18 for the description of the figure. The light chain sequences of the five Fabs individually designated DL 41 19, DO 41 11, GL 41 1, MT 41 12 and SS 41 8 have been assigned the respective SEQ ID NOs 147, 148, 149, 150 and 151.

Please amend the paragraph starting at page 16, line 32, to read as follows:

Figures 27A through 27E illustrate the nucleotide sequence of the b12 heavy chain VH and constant regions in the pEe6HC BM12

mammalian expression vector as described Example 4d. The amino acid residue sequence of the b12 heavy chain VH is given. The b12 VH has been modified for expression in mammalian cells as described in Example 4d.

Please amend the paragraph starting at page 17, line 23, to read as follows:

Figure 29A through 29S illustrates the nucleotide sequence of the pEE12 mammalian expression vector and the b12 IgG1 heavy and light chain genes, pEe12 Combo BM 12, as described in Example 4f. The VH and light chain genes have been modified for expression in mammalian cells as described in Example 4.

Please amend the paragraph starting at page 25, line 15, to read as follows:

A preferred human monoclonal antibody of this invention has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group of sequences consisting of SEQ ID NOs 66, 67, 68, 70, 72, 73, 74, 75, 78 and 79, and conservative substitutions thereof.

Please amend the paragraph starting at page 34, line 35, to read as follows:

Particularly preferred is the immunoglobulin IgG1 human antibody described herein that is comprised of the b12 antibody Fab fragment and human Fc domain derived from an IgG1 subtype,

designated b12 IgG1. The structure and preparation of this preferred human monoclonal antibody is described herein, and is prepared using the recombinant DNA expression vector pEE12. The complete nucleotide sequence of the vector for expression the complete heavy and light chains in the form of b12 IgG1 is shown in Figure 27 and also in SEQ ID NOs 156 and 170. Accordingly, the amino acid residue and nucleotide sequences, respectively, for a preferred complete heavy chain are shown in SEQ ID NOs 155 and 154, respectively, and for a preferred light chain are shown in SEQ ID NOs 153, and 152, respectively. The nucleotide sequences for preferred heavy and light chains are also shown in SEQ ID NOs 169 and 168, respectively.

Please amend the paragraph starting at page 57, line 21, to read as follows:

Sequence comparisons of identified HIV-immunoreactive monoclonal antibody variable chain region sequences are shown herein in Figures 10A and 10B through 13A and 13B. The sequences are aligned based on sequence homology, and groups of related antibody molecules are identified thereby in which heavy chain or light chain genes share substantial sequence homology.

Please amend the paragraph starting at page 69, line 34, to read as follows:

A particularly preferred vector of the present invention includes a polynucleotide sequence that encodes a heavy or light chain variable region of a human monoclonal antibody of the present invention. Particularly preferred are vectors that

include a nucleotide sequence that encodes a heavy or light chain amino acid residue sequence shown in Figures 10A and 10B through 13A and 13B, that encodes a heavy or light chain having the binding specificity of those sequences shown in Figures 10A and 10B through 13A and 13B, or that encodes a heavy or light chain having conservative substitutions relative to a sequence shown in Figures 10A and 10B through 13A and 13B, and complementary polynucleotide sequences thereto.

Please amend the paragraph starting at page 111, line 30, to read as follows:

In addition, the ability of the recombinant human HIV-1 immunoreactive Fabs b3, b6, b11, b12, b13, and b14 to neutralize the HXBc2 molecular clone of gp120 derived from HTLV-IIIB (LAI) was determined in an envelope complementation assay. The supernatant containing recombinant HIV-1 virions from cotransfected COS-1 cells was incubated with the recombinant Fabs prior to incubation with Jurkat cells. The recombinant HIV-1 virions contained the HXBc2 clone of HIV-1 strain LAI which encodes a chloramphenicol acetyltransferase (CAT) gene. Upon infection of Jurkat cells with the recombinant HIV-1 virions, the CAT gene was expressed and CAT activity measured. Activity of the CAT gene was therefore an indication of infectivity of the Jurkat cells with the recombinant HIV-1 virion. Lack of CAT activity indicated the Jurkat cells were not infected with the recombinant HIV-1 virion.

Please amend the paragraph starting at page 121, line 8, to read as follows:

First, the b12 VH region was cloned into a pSG-5 expression vector (Green et al., Nucl. Acids Res., 16:369 (1988)) to fuse the b12 VH to the heavy chain constant domains (CH1, CH2, and CH3) of an IgG1 antibody molecule. The double-stranded Fab b12 DNA was used as a template for isolating the gene encoding the VH region of the Fab b12, the amino acid residue sequence of which is listed in SEQ ID NO 66. Fab b12 DNA and mouse B73.2 IgG1 DNA (Whittle, et al., Protein Eng., 1:499 (1987) and Bruggmeman, et al., J. Exp. Med., 166:1351 (1987)) were used as templates for a PCR amplification for the construction of a DNA fragment consisting of the unique Kozak sequence for the control of heavy chain expression, the mouse B72.3 heavy chain leader sequence (MEWSWVFLFFLSVTTGVHS (SEQ ID NO 155 from amino acid residue sequence 1 to 20)), the human VH consensus sequence (QVQLVQ (SEQ ID NO 155 from amino acid residue sequence 21 to 26)), and the VH region of the Fab b12. Altering the beginning of the VH from the mouse consensus sequence to the human consensus sequence also destroyed the original Xho I cloning site. The restriction sites EcoR I and Sst I were introduced in the amplification reaction and were located at the 5' and 3' ends of the fragment, respectively. The procedure for creating the modified VH fragment by combining the products of the two separate PCR amplifications is described below.

Please amend the paragraph starting at page 121, line 34, to read as follows:

The primer pair, HC-1 (SEQ ID NO 157) and HC-2 (SEQ ID NO 158) as shown in Table 10, was used in the first PCR reaction to amplify a portion of the Fab b12 VH gene and incorporate the

human heavy chain consensus sequence into the 5' end of the VH fragment and introduce an Sst I cloning site in the 3' end of the VH fragment. In addition, the 5' PCR primer introduces sequences into the VH fragment which form 27 base pairs of homology with the mouse leader sequence fragment prepared below. The 27 base pairs of homology in the fragments is used in a subsequent PCR reaction to fuse the two PCR products (Yon and Fried, Nucl. Acids Res., 17:4895 (1989)) to form a modified VH fragment consisting of the EcoR I cloning site, the mouse leader sequence 72.3, the human consensus sequence, the remaining VH coding sequence, and the Sst I cloning site. For the PCR reactions, 1 µl containing 100 ng of Fab b12 DNA was admixed with 10 µl of 10X PCR buffer in a 0.5 ml microfuge tube. To the DNA admixture, 8 µl of a 2.5 mM solution of dNTPs (dATP, dCTP, dGTP, dTTP) was admixed to result in a final concentration of 200 micromolar (µM) of each dNTP. 1 µl (equivalent to 20 picomoles (pM)) of the 5' forward HC-1 primer and 1 µl (20 pM) of the 3' backward HC-2 primer were admixed into the DNA solution. To the admixture, 73 µl of sterile water and 2.5 units of Taq DNA polymerase was added. Two drops of mineral oil were placed on top of the admixture and 35 rounds of PCR amplification in a thermocycler were performed. The amplification cycle consisted of 52°C for 1 minute, 72°C for 2 minutes and 94°C for 0.5 minutes.

Please amend the paragraph starting at page 122, line 31, to read as follows:

The primer pair, HC-3 (SEQ ID NO 159) and HC-4 (SEQ ID NO 160) as shown in Table 10, was used in a separate PCR reaction to amplify the mouse B72.3 leader sequence and incorporate an EcoR I

cloning site at the 5' end of the fragment and to introduce a 27 base pair sequence which has homology to the modified VH fragment prepared above. Double-stranded DNA encoding the mouse B73.2 IgG1 (Whittle, et al., supra) was used as a template for preparation of the mouse 72.3 leader sequence. The PCR reaction to prepare the mouse leader sequence fragment was performed using the same conditions as described above for the preparation of the modified VH fragment.

Please amend the paragraph starting at page 123, line 15, to read as follows:

A third PCR amplification using the primer pairs, HC-1 (SEQ ID NO 157) and HC-3 (SEQ ID NO 159) as shown in Table 10, was performed to fuse the mouse leader fragment with the modified VH fragment. The primers used for this amplification were designed to preserve an EcoR I site, a unique Kozak sequence, and the mouse B72.3 heavy chain leader sequence on the 5' end of the amplified fragment and to preserve the Sst I cloning site on the 5' end of the amplified fragment. The templates used in this PCR reaction were the two purified PCR reaction products described above. The PCR reaction and subsequent purification of the PCR product were performed as described above.

Please amend the paragraph starting at page 124, line 7, to read as follows:

The primer pair, HC-1 (SEQ ID NO 157) and HC-6 (SEQ ID NO 162) as shown in Table 10, was used in the first PCR reaction to preserve the 5' region of the modified b12 heavy chain fragment

and destroy the Bgl II restriction site at amino acid residue 87 of the heavy chain. The HC-6 primer introduces sequences into the VH fragment which form 32 base pairs of homology with the remaining portion of the VH fragment which will be prepared as described below. The 32 base pairs of homology in the fragments was used in a subsequent PCR reaction to fuse the two PCR products (Yon and Fried, supra) to form a modified VH fragment as described above but without the Bgl II restriction site. The PCR reaction was performed and the PCR products were purified as described in Example 4a1.

Please amend the paragraph starting at page 125, line 2, to read as follows:

A third PCR amplification using the primer pairs, HC-1 (SEQ ID NO 157) and HC-3 (SEQ ID NO 159) as shown in Table 10, was performed to fuse the two VH fragments in which the Bgl II restriction site had been destroyed. The primers used for this amplification were designed to preserve an EcoR I site, a unique Kozak sequence, and the mouse B72.3 heavy chain leader sequence on the 5' end of the amplified fragment and the Sst I cloning site on the 3' end of the amplified fragment. The templates used in this PCR reaction were the two purified PCR reaction products described above. The PCR reaction and subsequent purification of the PCR product were performed as described in Example 4a1.

Please amend the table starting at page 126, line 1, to read as follows:

Table 10

SEQ ID NO	Primer		
(157)[1]	HC-1	(F)	5' CAGGTTCAGCTGGTTCAGTCCGGGG CT 3'
(158)[2]	HC-2	(B)	5' CCTTGGAGCTCACGATGACCGTGGT TCCTTGGCCCCAGACGTCC3'
(159)[3]	HC-3	(F)	5' GGCCGCGAATTCGCCGCCACCATGG AATGGAGCTGGGTCTTTCTCTTCTT CCTGTCAGTA 3'
(160)[2]	HC-4	(B)	5' AGCCCCGGACTGAACCAGCTGAAC CTG 3'
(161)[4]	HC-5	(F)	5' GGAGTTGAGGAGCCTCAGGTCTGCA GACACGG 3'
(162)[4]	HC-6	(B)	5' CCGTGTCTGCAGACCTGTGGCTCCT CAACTCC 3'
(163)	LC-1	(F)	5' GATGCCAGATGTGAGATCGTTCTCA CGCAGTCT 3'
(164)[3,5]	LC-2	(B)	5' GCGGGATCCGAATTCTCTAGAATTA ACACTCTCCCCTGTTGAAGCTCTTT GTGACGGGCGAACTCAG 3'
(165)[3]	LC-3	(F)	5' GCGCGAATTCACCATGGGTGTGCCC ACTCAGGTCCTGGGGTTGCTGCTGC 3'
(166)	LC-4	(B)	5' AGACTGCGTGAGAACGATCTCACAT CTGGCATC 3'
(167)[6]	LC-5	(F)	5' GCGCAAGCTTACCATGGGTGTGCCC ACTCAGGTCCTGGGGTTGCTGCTGC 3'

Please amend the paragraph starting at page 129, line 7, to read as follows:

The b12 light chain was cloned into a separate pSG-5 expression vector (Green et al., supra). The double-stranded Fab b12 DNA was used as a template for isolating the gene encoding the light chain of the Fab b12, the amino acid residue sequence the light chain of Fab b12 is listed in SEQ ID NO 97. Mouse B73.2 IgG1 DNA (Whittle, et al., Protein Eng., 1:499 (1987) and Bruggmeman, et al., J. Exp. Med., 166:1351 (1987)) was used as a template for isolating the mouse B73.2 leader sequence. Fab b12 and mouse B73.2 IgG1 DNA were thus used as templates for a PCR amplification for the construction of a DNA fragment consisting of the unique Kozak sequence for control of light chain expression, the mouse B72.3 light chain leader sequence (MGVPTQLGLLLWLTDARC (SEQ ID NO 153 from amino acid residue sequence 1 to 20)), and the b12 light chain beginning with a human light chain amino acid consensus sequence (EIVLTQSP (SEQ ID NO 153 from amino acid residue sequence 21 to 28)). Altering the beginning of the light chain from the mouse amino acid consensus sequence to the human amino acid consensus sequence also destroys the original Sac I cloning site. The restriction site, EcoR I, was introduced in the amplification reactions and was located at both the 5' and 3' ends of the fragment. The procedure for creating this fragment by combining the products of two separate PCR amplifications is described below.

Please amend the paragraph starting at page 129, line 32, to read as follows:

The primer pair, LC-1 (SEQ ID NO 163) and LC-2 (SEQ ID NO 164), was used in the first PCR reaction as performed above to amplify the Fab b12 light chain gene and incorporate the human light chain consensus sequence into the fragment and the EcoR I cloning site into the 3' end of the b12 light chain fragment. For the PCR reaction, 1 µl containing 100 ng of Fab b12 DNA was admixed with 10 µl of 10X PCR buffer in a 0.5 ml microfuge tube. To the DNA admixture, 8 µl of a 2.5 mM solution of dNTPs (dATP, dCTP, dGTP, dTTP) was admixed to result in a final concentration of 200 µM of each dNTP. 1 µl (equivalent to 20 pM) of the LC-1 primer and 1 µl (20 pM) of the 3' backward LC-2 primer was admixed into the DNA solution. To the admixture, 73 µl of sterile water and 2.5 units of Taq DNA polymerase was added. Two drops of mineral oil were placed on top of the admixture and 35 rounds of PCR amplification in a thermocycler were performed. The amplification cycle consisted of 52°C for 1 minute, 72°C for 2 minutes and 94°C for 0.5 minutes.

Please amend the paragraph starting at page 130, line 17, to read as follows:

The primer pair, LC-3 (SEQ ID NO 165) and LC-4 (SEQ ID NO 166) as shown in Table 10, was used in a separate PCR reaction to amplify the mouse light chain B72.3 leader sequence and incorporate an EcoR I cloning site at the 5' end of the fragment and to introduce a 27 base pair sequence which has homology to the modified light chain fragment prepared above. Double-stranded DNA encoding the mouse B73.2 IgG1 (Whittle, et al., supra) was used as a template for preparation of the mouse 72.3 leader sequence. The PCR reaction to prepare the mouse leader

sequence fragment was performed using the same conditions as described in Example 4a for the preparation of the modified VH fragment.

Please amend the paragraph starting at page 131, line 3, to read as follows:

A third PCR amplification using the primer pairs, LC-1 (SEQ ID NO 157) and LC-4 (SEQ ID NO 166) as shown in Table 10, was performed to fuse the light chain mouse leader fragment with the modified light chain fragment. The primers used for this amplification were designed to preserve an EcoR I site, a unique Kozak sequence, and the mouse B72.3 light chain leader sequence on the 5' end of the amplified fragment and to preserve the EcoR I cloning site on the 5' end of the amplified fragment. The templates used in this PCR reaction were the two purified PCR reaction products described above. The PCR reaction and subsequent purification of the PCR product were performed as described in Example 4a1.

Please amend the paragraph starting at page 135, line 35, to read as follows:

The primer pair, LC-5 (SEQ ID NO 167) and LC-2 (SEQ ID NO 165), was used in the PCR reaction as described in Example 4a1 to amplify the Fab b12 light chain gene and incorporate HindIII and EcoR I cloning sites into 5' and 3' ends of the fragment, respectively. The b12 pSG-5 vector containing the b12 light chain was used as the template in the PCR reaction. For the PCR reaction, 1 µl containing 100 ng of b12 pSG-5 DNA was admixed

with 10 µl of 10X PCR buffer in a 0.5 ml microfuge tube. To the DNA admixture, 8 µl of a 2.5 mM solution of dNTPs (dATP, dCTP, dGTP, dTTP) was admixed to result in a final concentration of 200 micromolar (µM) of each dNTP. 1 µl (equivalent to 20pM) of the LC-5 primer and 1µl (20 pM) of the 3' backward LC-2 primer was admixed into the DNA solution. To the admixture, 73 µl of sterile water and 2.5 units of Taq DNA polymerase was added. Two drops of mineral oil were placed on top of the admixture and 35 rounds of PCR amplification in a thermocycler were performed. The amplification cycle consisted of 52°C for 1 minute, 72°C for 2 minutes and 94°C for 0.5 minutes.

Please amend the paragraph starting at page 137, line 19, to read as follows:

The heavy chain cassette was removed from the pEE6 vector by digestion with BglII and Sal I. The pEE12 vector containing the light chain gene, prepared in Example 4e, was also digested with BglII and Sal I. The heavy chain cassette and the pEE12 vector containing the light chain gene from Example 4e were ligated together at the BglII and Sal I sites as described in Example 4d. The combinatorial construct was transformed into DH5α competent cells and miniprep DNA was analyzed for the presence of the heavy and light chains as in Example 4d. The nucleotide sequence of the heavy and light chain genes was determined. The nucleotide sequence of pEe12 Combo BM 12, the pEE12 vector containing the b12 heavy and light chain genes is given in the sequence listing as SEQ ID NO 156 and is illustrated in Figures 29A through 29S.

Please amend the paragraph starting at page 150, line 18, to read as follows:

The results have implications for passive immunization and vaccine design. The ability of b12 IgG1 to neutralize a range of primary isolates implies conservation of a structural feature associated with the CD4 binding site of gp120 which is accessible to antibody and important for neutralization. A vaccine might seek to present this feature to the immune system. Clearly, the feature is present on recombinant gp120 since b12 was affinity selected from a library using this molecule. However, b12 and related antibodies formed only a small part of the repertoire affinity selected from this library by recombinant gp120. Most of the antibodies obtained were far less potent in neutralization even though they were also directed to the CD4 binding site, were cross-competitive with b12 for binding to recombinant gp120 and had similar affinities to b12 (Barbas et al., Proc. Natl. Acad. Sci., U.S.A., 89:9339-9343 (1992), Barbas et al., J. Mol. Biol., 230:812-823 (1993), and Example 2b6)(c). Therefore, recombinant gp120 appears to present the b12 epitope in conjunction with several other weakly neutralizing and overlapping epitopes and its efficacy as a vaccine may suffer. Interestingly, evidence from antibody binding to infected cells suggests that b12 does recognize a native conformation of gp120 more effectively than other CD4 binding site antibodies (Example 7). In any case, b12 IgG1 and the library approach could be useful in vaccine and passive immunization evaluation. The ability of a candidate vaccine to preferentially bind b12 and/or preferentially select potent neutralizing antibodies from libraries should be positive indicators for vaccine development.

Please amend the paragraph starting at page 152, line 8, to read as follows:

Competitive ELISAs were performed between the Fabs b3, b6, b11, b12, and b14 and the b13 whole IgG1 antibody. The whole antibody was obtained by splicing constant domain genes with the b13 Fab and expressing the protein in Chinese Hamster Ovary cells (CHO) as described in Example 4 (Bender et al., supra and in Example 4a for the Fab b12). The ELISA was performed as described above in Example 2b6b. Briefly, microtiter wells were coated with 0.1 µg/ml of gp120 derived from the HIV-1 strain LAI in 0.1 M bicarbonate buffer at pH 8.6. Soluble or free Fab fragments were serially diluted from 1:100 to 1:32,000 in 0.5% BSA/0.025% Tween 20/PBS. The dilution of b13 IgG1 was held constant at 1:10,000 in 0.5% BSA/0.025% Tween 20/PBS. The b13 IgG1 and Fabs were admixed, added to the gp120-coated microtiter wells and maintained for 120 minutes at 37°C. After maintenance, the wells were carefully washed ten times with 0.05% Tween 20/PBS. The amount of b13 IgG1 antibody bound to the plate after washing was detected using a peroxidase-labeled antibody specific for the Fc portion of IgG1 contained on the b13 antibody.

Please amend the paragraph starting at page 168, line 35, to read as follows:

V_H and V_L domains of 32 gp120 clones were sequenced and the V_H domains compared using MacVector software. This analysis immediately established that a number of the clones, including those selected by panning against different antigens, are closely related to one another. The exception to this is the Fabs

selected by panning against the V3 loop peptide which are not related to the Fabs selected by panning against the gp120/160 antigens. Figures 10A and 10B show that the V_H sequences derived from gp120/160 panning can be organized into 7 groups. The broad features apparent from a comparison of amino acid sequences are discussed herein.

Please amend the paragraph starting at page 170, line 18, to read as follows:

The V_L sequences of the Fabs were organized into the groups defined in Figures 10A and 10B are shown in Figures 11A and 11B. Immediately apparent was the extensive chain promiscuity as evidenced by the pairing of different light chains with the same or a very similar heavy chain with retention of antigen binding capability and indeed, for the most part, antigen affinity as compared with Figures 10A and 10B. This promiscuity can be explored further by reference to the groups considered above.

Please amend the paragraph starting at page 171, line 27, to read as follows:

In summary, the heavy chain (V_H) sequences was organized into 7 groups where each member of a group has an identical or very similar CDR3 region with a limited number of differences elsewhere. When the light chains (V_L) were constrained into the groupings defined by their heavy chain partners, considerable light chain sequence variation was observed. This phenomenon of chain promiscuity has been observed previously and can be appreciated by reference to Figures 11A and 11B. Marked

neutralizing ability was confined to two groups of sequences. The first group consisted of Fabs 4, 7, 12 and 21 which have very similar heavy and light chains. The second group consisted of Fabs 13, 8, 18, 22 and 27. Only Fab 13 showed marked neutralizing ability, although the others showed some weaker activity. Interestingly in this group Fab 13 did have a light chain distinct from the other members of the group.

Please amend the paragraph starting at page 172, line 13, to read as follows:

To further explore possible functional heavy-light chain combinations, the heavy chain of clone b12 (also referred to as Fab 12 for the corresponding soluble Fab preparation) shown in Figures 10A and 10B was recombined with the original light chain library prepared in Example 2 to construct a new library H12-LCn. In addition, the b12 light chain was recombined with the original heavy chain library to construct a library Hn-L12. These two libraries were taken through 3 rounds of panning against gp120 (IIIB) as described in Example 2b5). The Fabs expressed from the resultant immunoreactant clones were analyzed as described in Example 3 above. Clone b12 was chosen as this Fab neutralized HIV-1 <u>in vitro</u> as shown in Example 3.

Please amend the paragraph starting at page 173, line 17, to read as follows:

The sequences of the light chains which bound to the b12 heavy chain clone are shown in Figures 12A and 12B. The sequences are compared to the sequence for the original light

chain from clone b12. The light chains are identified by numbers which do not correspond to the original light chain clones; the assigned numbers of the newly selected clones having new light chains are thus arbitrary. The sequences of these light chains are also listed in the Sequence Listing from SEQ ID NO 114 to 122. Some light chain sequences are identical. In addition to immunoreactivity with gp120, the new Fabs isolated from these shuffled clones were tested in the syncytia assay for neutralization of HIV-1 infection as described in Example 3. Four shuffled monoclonal Fab antibodies, each having the heavy chain from clone b12, a known HIV-1 neutralizing clone, and new light chains designated L28, L25, L26 and L22, all exhibited approximately 60% neutralization in a syncytia assay with 0.4 µg/ml purified Fab. This effect was equivalent to that obtained with the original clone b12 heavy and light chain pair. Maximum neutralization of approximately 80% was obtained with the H12/L28 and H12/L25 Fabs at 0.7 µg/ml which was equivalent to that seen with the original clone b12 heavy and light pair. The neutralization resulting from the H12/L22 and H12/L26 Fabs plateaued at 60% with Fab concentrations of 0.4 µg/ml up to 1.0 µg/ml. Thus, in addition to the gp120 immunoreactive and HIV neutralizing Fabs obtained in the original library prepared as described in Example 2, by shuffling a known neutralizing heavy chain with a library of light chains, new HIV-1 neutralizing Fab monoclonal antibodies have been obtained.

Please amend the paragraph starting at page 174, line 26, to read as follows:

The sequences of the heavy chains which bound to the b12

light chain clone are shown in Figures 13A and 13B. The sequences are compared to the sequence for the original heavy chain from clone b12. The heavy chains are identified by numbers which do not correspond to the original light chain clones; the assigned numbers of the newly selected clones having new heavy chains are thus arbitrary. The sequences of these light chains are also listed in the Sequence Listing from SEQ ID NO 123 to 132. Some light chain sequences are identical. In addition to immunoreactivity with gp120, the new clones were tested in the syncytia assay for neutralization of HIV-1 infection as described in Example 3. Two shuffled monoclonal Fab antibodies, each having the light chain from clone b12, a known HIV-1 neutralizing clone, and new heavy chains designated H2 and H14, exhibited approximately 40% neutralization in a syncytia assay with 1.0 and 0.5 µg/ml purified Fab, respectively. This effect was equivalent to that obtained with the original clone b12 heavy and light chain pair at a concentration of 2 µg/ml. Maximum neutralization of approximately 50% was obtained with the Fab having the new H14 chain at 1.0 µg/ml compared to 80% neutralization with 0.7 µg/ml with the original clone b12 heavy and light pair. Thus, in addition to the gp120 immunoreactive and HIV neutralizing Fabs obtained in the original library prepared as described in Example 2, by shuffling a known neutralizing light chain with a library of heavy chains, new HIV-1 neutralizing Fab monoclonal antibodies have been obtained.

Please amend the paragraph starting at page 178, line 5, to read as follows:

Whatever the arguments about light chain authenticity, the

heavy chains of Figures 10A and 10B present many features of interest. The most frequently used heavy chain is of the clone b8 type. It could be argued that this usage simply represents bias in PCR amplification. However, the occurrence of approximately equal numbers of clones in this group amplified by VH1a and VH3a primers argues against this notion. Furthermore, the existence of intraclonal variants in some groups indicates that one is at least sampling different genes from the initial library.

REMARKS

The amendments to the specification are to provide corrections to obvious typographical errors, and to correct sequence identifications. The amendments also insert revised Figure labels where appropriate to correspond to the required changes in the Figures themselves. The amendments further correct informalities in the use of sequence identifiers by assigning SEQ ID NOs to the second strand of nucleotide sequence where double-stranded nucleotide sequences are given in the Figures. Support for changing sequence identifiers is found at the corresponding number in the Sequence Listing. Attached hereto is the Appendix with markings showing the changes made to the specification.

The change of the number "97" to "79" at page 25 is to correct a typographical transposition of the numbers, support for which change is at page 25, lines 22-27 showing that SEQ ID NO 97 is a light chain and not a heavy chain sequence as listed above.